THE WALTER AND ELIZA HALL INSTITUTE OF RESEARCH IN PATHOLOGY AND MEDICINE

TWELFTH ANNUAL REPORT 1930-31

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Mr. Lort Smith, who has represented the Walter and Eliza Hall Trustees on the Board of the Institute since its inception, and who has acted as Treasurer during this period, died after a short illness on April 6th. His loss will be keenly felt, and we shall miss greatly his personal interest in the progress of the Institute.

The Twelfth Annual Report

OF THE

Walter and Eliza Hall Institute of Research

July, 1931.

The financial conditions prevailing during this year have necessarily prevented any expansion of the work of the Institute. During the three preceding years substantial help has been forthcoming from the Commonwealth Department of Health, but this year no further grant was available. By the exercise of rigid economy it was possible to spread last year's subsidy over the early months of 1931, and we have been able to carry on the researches originated under previous grants. A grant of £600 has been made available for 1931-32, which will cover the salary of Mr. Tom Eades and the expenses of venom collection, so that the preparation of anti-sera at the Commonwealth Serum Laboratories can be continued. A grant of £100 has also been provided to defray the cost of further work on anterior poliomyelitis.

The maintenance of the biochemical department has entailed a considerable expenditure from the general fund, and retrenchment in salaries and wages of the whole Institute staff has been necessary. We have also had to reduce our purchases of materials and apparatus, and this has to some extent hampered our work.

Further extensive reduction, now coming into effect, of salaries and other expenditure should enable us to remain solvent and continue the researches upon which we are engaged, but some loss of efficiency and diminution of output will be inevitable.

The Work of the Institute.

Australian Snake Venoms.

Our thanks are due to the President, Council and Director of the Zoological Gardens, whose friendly co-operation has enabled us to maintain our collection of reptiles at the Zoological Gardens and to retain Mr. Tom Eades' services for their collection and care.

During the season collecting trips have been made to Mount Gambier, Penshurst, Terang, Pirron Yallock (2), Tallangatta, and South-Western Queensland.

This year we have collected or purchased 33 death adders, 193 tiger snakes, 143 copperheads, 29 black snakes, and 5 brown snakes. Mr. Fleay has kindly given us several specimens of *Denisonia coronoides* (the white lipped snake), a brown snake and a black snake. One example of *Denisonia nigrescens* has also come into our possession. Examples of *Furina annulata* (the bandy bandy) and of two rare Denisonias, *D. signata* and *D. suta,* together with three further specimens of *D. maculata,* were captured in South-West Queensland by Mr. Eades.

Of the venoms, we have obtained this year 3.6 grammes of death adder, 19.8 grammes of tiger, 14.5 grammes of black tiger snake, 9.1 grammes of copperhead, 4.4 grammes of black snake, and 60 milligrammes of brown snake venom.

We purchased from Mr. Murray, who collected them for us in Chappell Island, Bass Strait, a fine collection of black tiger snakes, over 50 in number. We are investigating this sub-species from the standpoint of its venom yields and the toxicity of its venom, and have found that this venom can be neutralised, though not in the same proportions as the venom of the tiger snake, by tiger snake antivenene prepared at the Commonwealth Serum Laboratories.

Antivenene prepared against tiger snake venom is now generally available, and a number of cases of snake bite has been treated with it with highly satisfactory results.

The Serological and Blood Relationships of Some Australian Snakes.

We have attempted to ascertain whether these relationships can be utilised to confirm morphological findings in the differentiation of closely allied species of venomous

snakes, and have explored the specificity of the precipitin and complement fixation reactions using the sera of nine species of Australian snakes and antisera prepared in rabbits against seven of them.

Neither of these methods in its simple form permits of the differentiation of species even when these belong to different genera of the *colubridae.* The results of the precipitin tests confirmed Graham Smith's findings, and were widely non-specific, especially when strong antisera were used.

The complement fixation test was somewhat more specific. The antisera invariably fixed more complement with the serum used in its preparation than with the sera of other species, and the results were in accord with the zoological order of the genera. In two cases studied the results were of no value in allotting a species to its genus.

By the use of absorption, against the application of which to the precipitin test there are strong theoretical objections, we were able somewhat irregularly to obtain antisera which reacted in precipitin tests with only one of the sera tested.

Hæmagglutination does not appear to be much more specific than the precipitin and complement fixation tests. The chief advantage of this method lies in the fact that complement fixation can be applied to absorbed sera since the excess of absorbing substance can readily be removed.

Further studies are in progress to test the value of this last method, using some additional species of Australian snakes.

Observations on Pseudechis australis.

Mr. Thomson has been able to clear up the confusion which has existed in regard to several of the species in the genus *Pseudechis* by showing that *P. australis* (Gray), *P. darwiniensis* (Macleay), and *P. cupreus* (Boulenger) are synonymous.

The differentiation of *P. darwiniensis* from *P. australis* depended upon the proportions of the frontal shield. It was found that this character is not uniform, since a series of specimens of *P. australis* from Cape York Peninsula and another of supposed *P. darwiniensis* from the Macleay Museum showed gradations in this character, and variation of similar extent was found within a well defined species of this genus—*P. porphyriacus.*

Boulenger's description of *P. cupreus* was based partly on an incomplete description by Krefft (1865) of a specimen from the Murray and partly on a specimen figured by McCoy and described by him in 1887 as *P. australis*. The first specimen was completely described by Krefft in 1868, and fits accurately with *P. australis*, and the second proved on examination to be a *Demansia* with 10 post maxillary teeth.

This large snake, *P. australis*, is common in Cape York Peninsula, where it frequents well grassed and swampy country and takes refuge under ground.

The venom yields of a single live specimen, milked at weekly intervals for six months, varied from 311 to 86 milligrammes of dry venom, the lowest figure being obtained just after capture, during which much had been lost. The highest yield was obtained just after the snake had sloughed his skin and a week after being fed. The lowest yields were obtained when the snake was about to cast its skin. McLennan's two samples, earlier examined, which were obtained by milking specimens immediately after death, weighed 0.2 grammes and 0.358 grammes respectively.

The venom proved to be less toxic than that of the tiger snake, brown snake, death adder, copperhead, or of the giant brown snake (*Oxyuranus maclennani*). It was of about the same order of toxicity as that of two other snakes in the same genus *P. porphyriacus* and *P. guttatus*. Like these, it is powerfully hæmolytic and feebly neurotoxic, but differs from them in the absence of thrombin, having on the contrary a powerfully anticoagulant action. Unlike most other Australian venoms, it causes systolic arrest of isolated heart muscle.

Of McLennan's two samples, one was obviously identical with the freshly collected venom from our living specimen and the other showed feebler hæmolytic power and exhibited marked hæmorrhagic effects in the larger doses required to cause death. It had been subjected to bacterial action during a wet season in the tropics and possessed a very high acidity.

Some evidence was presented, that samples of fresh dry venom obtained on different occasions from the same snake, may exhibit variation in potency.

Brown Snake Venom.

Dr. Kellaway has made some further observations on the toxicity of the venom of the common brown snake *Demansia textilis*. This snake, which has a poor biting apparatus, and which in captivity gives an average venom yield of only 2 milligrammes of dry venom, causes an appreciable mortality. According to Hamilton Fairley, 8.6 per cent. of persons bitten by this snake succumb. This mortality is explained by the high toxicity of the venom, which is only a little inferior to that of the tiger snake. It contains a powerful thrombin and neurotoxin, but has only feeble hæmotoxic power. The thrombin is of some interest, since it appears to be diffusible in both the sheep and the rabbit; it readily gains access to the bloodstream when injected subcutaneously or even intradermally, and thus often causes death by intravascular thrombosis. It is unstable, and is rapidly destroyed when dissolved in a saline solution of p.H. 3.8.

Observations upon the monkey and cat suggest that the neurotoxin of this venom like that of the death adder has some peripheral action.

The Specificity of Active Immunity to Snake Venoms and Some Observations on Cellular Immunity.

Earlier experiments upon guinea pigs immunised with a number of venoms had suggested that active immunity in this species was less specific than passive immunity conferred by the injection of monovalent antivenene.

Groups of rabbits, immunised against the respective venoms of the tiger snake, copper head and death adder, were found to exhibit a greater degree of non-specificity than could be accounted for by the action of circulating antibodies.

In the attempt to explain this phenomenon, it was shown that venoms, closely allied to that used for immunisation, could function as secondary stimuli in these immune animals, but it appeared doubtful whether the time necessary for this response was sufficient to account for the observed non-specific protection and certain that the time was insufficient when death adder venom was used for testing.

Splenectomy and "blocking of the reticulo-endothelial system" in some of these immune animals and in rabbits immunised with cobra venom yielded inconclusive results.

Those in rabbits immunised with tiger snake and cobra venom suggested that tissue immunity played no striking part, while those in rabbits immunised against death adder venom were consistent with the view that their immunity was not wholly dependent upon the presence of circulating antibody. This was supported by the relatively poor humeral antibody response of rabbits immunised with this venom and by the results of Dr. F. G. Morgan's experimental immunisation of horses.

Experiments on isolated auricles from rabbits actively immune to cobra venom confirmed Gunn and Heathcote's finding that perfused immune tissues exhibit greater resistance to the venom than normal rabbit tissues, but doubt was thrown on the validity of the perfusion experiment in the demonstration of cellular immunity, since it was shown that very small quantities of antibody protect the normal auricle *in vitro*.

The spermatozoa of normal rabbits are rapidly immobilised by cobra venom, but those from immune animals were found either to be hypersensitive or at least showed no striking immunity.

Publications.

KELLAWAY, C. H., and
WILLIAMS, F. ELEANOR:

"The Serological and Blood Relationships of Some Common Australian Snakes."

The Australian Journal of Experimental Biology and Medical Science (1931), 8, pp. 123-132.

KELLAWAY, C. H., and
THOMSON, D. F.:

"Observations on the Venom of a Large Australian Snake, *Pseudechis australis* (Gray)."

The Australian Journal of Experimental Biology and Medical Science (1930), 7, pp. 125-149.

KELLAWAY, C. H.:

"Observations on the Certainly Lethal Dose of the Common Brown Snake, *Demansia textilis*, for the Common Laboratory Animals."

(In the Press.)

KELLAWAY, C. H., and
WILLIAMS, F. ELEANOR:

"The Specificity of Active Immunity to Snake Venoms and Some Observations on Cellular Immunity."
(In the Press.)

The Mathison Lectures.

In November of last year, at the University of Melbourne, Dr. Kellaway delivered the Second Mathison Lectures. These, on the general subject of snake venoms and antitoxic immunity, were two in number, the first on the specificity of active immunity and the evidence for cellular immunity and the second on the immunity of the Australian snakes to their own venoms.

(i) The Specificity of Active Immunity to Snake Venom and the Evidence for Cellular Immunity.

In this lecture, the results of experiments on the specificity of active immunity to snake venoms in guinea pigs summarised in last year's report and of the further observations on rabbits, already summarised in the present report, were reviewed. It was concluded that active immunity is less specific than the passive protection afforded by the sera from immune animals and that this is only in part accounted for by the ability of closely allied venoms to act as secondary stimuli. Though no decisive evidence of immunity of the tissues of actively immune animals could be found, protection of these animals against venoms closely allied to that used for immunisation could not in all cases be accounted for by circulating humeral antibody. Finally, it was suggested that an additional factor in immunity might be the accumulation of antibody in the tissue spaces round those cells which were peculiarly liable to attack.

(ii) The Immunity of Australian Snakes to Their Own Venoms.

The Australian venomous snakes and to a less degree some non-venomous ones possess an extremely high immunity to Australian venoms, but are susceptible to the action of cobra venom. This immunity is in striking contrast to the susceptibility of mammalian species. Cold-bloodedness can be excluded as the prime cause of these differences.

If it could be shown that venom or its constituents were constantly or intermittently present in the circulating blood, this immunity would occasion no surprise, and various theories have been put forward to account for it in this way.

Waddel suggested that venom is absorbed from abrasions in the alimentary tract. This possibility has been denied on the ground that reptilian bile rapidly destroys venom. But destruction of venom by bile is inconsiderable and a portion of large doses of venom administered orally to snakes can be recovered from the excreta. In these experiments, however, there was no evidence that venom gained entry into the circulating blood.

Phisalix and Bertrand believed that the venom glands secrete venom into the circulating blood, and Calmette held that, since the toxic substance in snake plasma is heat labile, it is not venom itself, but some diastatic constituent of venom. The demonstration by numerous workers that the secretion of the homologous salivary gland in the non-venomous snakes is toxic and that the plasma is likewise toxic, is favourable to either of these hypotheses and either would serve to explain also the relatively high immunity of non-venomous snakes.

There are, however, many sera other than those of the ophidians which are toxic and which behave like snake sera in losing their toxicity when heated, antitoxic powers being unmasked. In some at least of these species there is no suggestion of a toxic secretion by the parotid gland. Further, much of the evidence upon which Phisalix and Bertrand's and Calmette's views are based is rendered suspect if the experimental reptiles behave like the Australian snakes which frequently bite themselves and their fellows in captivity, and so introduce venom into their plasma. In the Australian snakes immunity is to neurotoxin and thrombin, but no evidence could be found of neurotoxin or thrombin in the plasma of venomous snakes. The toxic action of snake sera was not masked by antivenene prepared against the homologous venom and the sera of rabbits immunised with venom failed to give positive immunological reactions with sera, and those immunised with sera failed to give positive immunological reactions with venom.

If, as Calmette found, sufficient reptilian serum be used, guinea pigs can be actively immunised against venom, but it is difficult to be certain that this result does not follow the injection of venom by bites during capture and in

captivity. Finally, in snakes from which the venom glands have been removed, there is no evidence of any progressive loss of toxicity of the plasma.

The third hypothesis has not previously been presented. It is that venom gains entry to the blood stream by self-inflicted bites or by the bites of other snakes, and that, of individuals exposed to an environment containing venomous species, only those have survived and propagated their kind, which were capable of resisting the action of venom. Upon this view the immunity of the hedgehog and doormouse to viper venom, as well as that of the venomous and non-venomous snakes, to various snake venoms is satisfactorily explained.

The nature of the immunity of venomous snakes was next considered. It was shown by perfusion experiments that the antitoxic power of the plasma was insufficient wholly to account for their immunity. When venom is injected intravascularly, the added toxicity of the plasma rapidly disappears, and this occurs when the Wolffian ducts are tied or the venom glands excised. It was further shown that the loss of toxicity was not due to destruction by enzyme action of the plasma. No evidence of excretion by the alimentary tract could be obtained. Absorption experiments with perfused snake tissues gave positive results, venom being lost or destroyed in contact with them *in vitro,* but perfused tissues from a highly sensitive mammal gave similar results. Perfusion experiments in which a limited quantity of Ringer's solution containing gum arabic and the washed suspended red blood corpuscles, was allowed to circulate naturally after removal by perfusion of most of the plasma, gave negative results. There was but little disappearance of added venom during the period available for experiment—2 to 3 hours. In some experiments, in which no venom was added, mobilisation of further antitoxic substances in the circulating fluid occurred during perfusion, though this result may possibly have been due to concentration in the diminishing bulk of circulating fluid. Concentration may also have neutralised the effect of absorption of venom by the tissues, water being absorbed faster than contained venom. Experiments in which in the intact snake the aorta and inferior vena cava were ligated at the cranial pole of the liver and venom was injected intravascularly into the anterior part of the reptile, seemed to exclude any detoxicating action of the liver upon the venom. Venom in doses of the order

of 20 milligrammes injected into the circulation of the anterior part of the snake caused no symptoms, but when injected into the vessels behind the ligatures rapidly took effect, causing paralysis of the poorly oxygenated tissues of the hinder end.

The most important factor in the immunity of snakes to their own and allied venoms is the high natural immunity of the tissues of the central nervous system, which allows them to withstand even high concentrations of venom for a considerable time. Finally, since even large doses of venom cannot act as specific secondary stimuli, the antitoxic immunity of snake plasma is unlikely to be of the same nature as that developed in mammals in the course of active immunisation.

Publications.

KELLAWAY, C. H.:

The Mathison Lectures—Snake Venoms and Antitoxic Immunity.

The Medical Journal of Australia, July 4th and July 11th, 1931.

The Physico-chemical Properties of Bacteriophages.

Dr. Burnet and Miss McKie have continued their work on physico-chemical differences amongst bacteriophages, in an effort to determine whether such differences can be correlated with the well marked functional differences described in several previous papers. This aim has not been accomplished, but in two directions interesting data have been obtained.

In studying the conditions of inactivation of bacteriophage by heat it was noted that the phage survived much better in Ringer solution than in saline. Following up this lead, the effect of various cations on bacteriophage was studied. It was found that as with living organisms in general there is an optimal ratio of Na: Ca for the survival of phage particles exposed to moderate heat. Different phages show very different degrees of resistance, but in all cases a ratio of about 50 Na: 1 Ca gave the highest percentage survival. The protective effect of calcium could also be shown in tests on the inactivating effects of certain dyes such as methylene blue and toluidine blue. In the presence of broth or of calcium salts no

inactivation occurred, but if the phage were diluted in normal saline, it was rapidly inactivated by weak solutions of the dye.

In discussing these phenomena, it was pointed out that the effect of calcium could be explained on the same basis as has been used to account for its action on animal and plant cells. Sodium and similar ions tend to favour permeability, uptake of water, excitability and final disintegration, the bivalent ions diminish permeability, excitability and the degree of hydration of a living surface. With so small a particle as a bacteriophage, analogous effects would manifest themselves chiefly in the degree of hydration and the apparent size. It was suggested that ionic influences of this sort must be kept in mind in any attempts to deduce the size of the bacteriophage particle.

A series of studies on the electrical behaviour of bacteriophages has also been carried out, using an agar electrode catophoresis vessel. The general finding that bacteriophages have a negative charge has been confirmed for a wide range of types. One type of phage showed an apparent positive charge, but on taking electroendosmotic phenomena into account, it was concluded that the phage particles actually possessed a very weak negative charge. It was possible to show that positively charged proteins were readily adsorbed to the bacteriophage particles by observing the change in the direction of their migration. Globin at p.H. 4.0 was particularly active, and, in addition, was capable of inactivating many phages at a low concentration.

Immunological Differences between Poliomyelitis Viruses.

In the last annual report, it was noted that Dr. Burnet and Dr. Jean Macnamara had observed one or two instances in which a typical attack of poliomyelitis had failed to protect a monkey against subsequent inoculation of another strain of the virus. The few monkeys still available were used to extend these observations. Although the number of cases is limited, clear evidence was obtained that a typical paralytic infection by a locally isolated virus does not result in immunity to a subsequent injection of the virulent Rockefeller M.V. strain and that the serum of such a monkey cannot neutralise the Rockefeller M.V. virus *in vitro,* although it does inactivate the local virus. Further, the only monkey that survived a paralytic infection by the Rockefeller virus was found to be non-resistant to the local virus.

These findings represent the most definite evidence of immunological difference between strains of poliomyelitis virus that had yet been recorded and they may have an important bearing on the epidemiology and serum treatment of the disease.

Staphylococcal Toxin.

The investigation of this toxin has continued to yield interesting results. It was found earlier that, by treatment with formalin, the toxic filtrates could be rendered innocuous to animals, while retaining their antigenic power. This phenomenon has been investigated in detail by Dr. Burnet. Staphylotoxin is inactivated much more rapidly than diphtheria toxin, the process taking a few hours instead of weeks. Detoxication takes a logarithmic course, and its rate is accelerated (a) by higher temperature, (b) by increased concentration of formaldehyde, (c) by a more alkaline reaction. All the characteristic activities of the toxin are diminished in almost parallel fashion. The atoxic material is actively antigenic, provoking good antitoxic titres when injected into rabbits. Two methods were devised for the estimation of the antitoxin binding power of the atoxic "anatoxin" *in vitro,* and by the use of these it was found that with complete detoxication the binding power fell constantly to half the value characteristic of the original toxin. The methods used for these estimations are subject to error, and not very accurate, but the results have been consistent with many batches of toxin and the 2 : 1 relationship between the binding powers of a toxin and its derivative anatoxin can probably be accepted as a genuine phenomenon.

This finding suggested that the reactions toxin-antitoxin and anatoxin-antitoxin are essentially stoichiometric in nature. Quantitative experiments along the classical lines of Arrhenius and Madsen were made to determine the course of partial neutralisation of toxin by antitoxin and by the use of mixtures of equivalent amounts of toxin anatoxin and antitoxin the extent of reversibility of the reaction was measured to a fair degree of accuracy.

The data from such experiments has been interpreted by adopting a new hypothesis of the nature of the toxin-antitoxin reaction. This assumes that the primary union is stoichiometric, resulting in an insoluble product on which the component in excess is adsorbed according to the ordinary adsorption equation. Such a view is capable of giving

a quantitative interpretation of all the data available for the staphylotoxin reactions and appears to be consistent with the published data for other toxins.

The Toxin-antitoxin Flocculation Test.

Following the general lines of the method devised by Ramon, it has been possible to show that staphylotoxin and anti-toxin show a zone of optimal precipitation. Like most of its other reactions, the precipitation appears rapidly and the optimal point is usually fairly distinct. The point of optimal flocculation is not identical with the neutral point as judged by hæmolytic tests, but bears a fairly constant relation to it. If the neutral ratio is represented by $\frac{T}{A} = 1.0$, the flocculating point corresponds to $\frac{T}{A} = 0.6$ approximately. In the case of diphtheria toxin it is usual to regard the flocculating ratio as indicating true neutrality, but with staphylotoxin this is certainly not the case, since the supernatant fluid from the most rapidly flocculating tube has always shown the amount of free antitoxin to be expected from the position of the neutral point as determined by hæmolytic tests.

By methods similar to those used by Glenny and Pope it has been found possible to recover both toxin and antitoxin from the washed floccules produced in the reaction, thus showing directly that the reaction is an indicator of toxin-antitoxin union.

Anatoxin, provided the action of formalin does not go beyond the point of complete detoxication, shows the same flocculation reactions as its toxin of origin. Under the conditions of such tests it also shows the same power to bind antitoxin as the original toxin. It seems, therefore, that the binding power of anatoxin varies with the conditions. If the original toxin is given the value 1 for each of its measurable characteristics, an "undamaged" anatoxin shows the following values: Hæmolytic titre 0, Binding power in dilute solution 0.5, Binding power in concentrated solution and flocculating power 1.0.

The effect of heat (100° C.) on staphylotoxin has been studied. Binding power and hæmolytic titre fall progressively, but at different rates. The flocculation point shifts considerably within a few minutes and then remains constant despite further heating.

Mr. Holden and Miss Freeman are collaborating in a series of preliminary experiments to explore the possibility of studying the toxin-anatoxin transformation along chemical lines. It has been found that the toxin may be precipitated by ice cold alcohol or acetone and recovered almost quantitatively from the precipitate, and it is hoped to develop a method for obtained purified preparations of toxin along these lines.

Immunity to Staphyloccal Toxin in Human Beings.

Dr. Lucy Bryce and Dr. Burnet have made a study of the development of natural antitoxic immunity in the human being and a parallel investigation on the rat is in progress. Both species show in the adult the frequent presence to staphylococcal antitoxin in the serum.

In man there is a passive transference of antitoxin from mother to infant. Through the courtesy and collaboration of the staff of the Queen Victoria Hospital, it has been possible to compare the cord blood of a large number of new-born infants with the blood of the mother. In a large proportion of the cases the two sera showed an identical content of antitoxin. In the first few months of life the antitoxic titre falls rapidly and is at a low level from 6-18 months. The average titre rises during childhood, and from the figures so far available the greatest rise seems to be in the period from 3-5 years. In old age the titre appears to fall somewhat, but sufficient material is not yet available to make certain that this fall is significant.

It seems reasonable to regard this course as entirely analogous to the development of antitoxic immunity to the diphtheria bacillus or the hæmolytic streptococci as judged by the Shick and Dick tests. There is the same primary passive immunity disappearing in a few months and followed by an active immunity, engendered in most cases by sub-infective contact with the toxigenic organism. The opportunities of contact with toxigenic staphylococci (almost ubiquitous skin organisms) naturally occur earlier than exposure to diphtheria or scarlet fever carriers and antitoxic immunity is developed at a considerably earlier average age.

A study of the age incidence of acute staphylococcal osteomyelitis suggests that one group of cases is associated with the period during which the circulating antitoxin is at a minimum.

Treatment of Infantile Dysentry by Bacteriophage.

In collaboration with Dr. Ian Wood and the staff of the Children's Hospital, a study of the response of cases to the administration of a highly active Flexner bacteriophage has been made by Miss McKie.

The polyvalent phage was grown on strains isolated from cases at the Children's Hospital the previous summer, and was highly active against all Flexner strains. It completely cleared W, VZ and Z cultures, these cultures remaining clear indefinitely. It included d'Herelle's Bacte-intesti and Bacte-dysenteri phages and a polyvalent phage from Rangoon as well as 20 or 30 strains of local origin, including representatives of all the types previously described.

All cases in one ward were treated with phage, and as controls, the cases in another ward remained untreated. Before phage was given, a stool specimen was tested for the presence of Flexner bacilli and for the presence of naturally occurring bacteriophages. Two ampoules (about 2 c.cms.) were given the first day and one ampoule on each of the two following days. Since bacteriophage acts best at a p.H. of about 8.0, attempts were made to keep the intestinal contents slightly alkaline, but owing to the high carbohydrate diet, these methods were unsuccessful. However, in spite of the acid reaction of the stools, fæcal filtrates always contained phage after it had been given by mouth to patients suffering from Flexner dysentery. Analysis of the results is not yet complete, but it is clear that, although no ill effects of any kind resulted from the treatment, the bacteriophage had little or no effect on the duration of the disease or on the death rate.

Types of Flexner bacilli isolated. As usual, Flexner W. strains (68.5%) predominated. A number of "inagglutinable" strains of Flexner bacilli were isolated, and these appear to form a homogeneous group. Their phage reactions and serology are being worked out.

Publications.

BURNET, F. M., and
MCKIE, M.

"Balanced Salt Action as Manifested in Bacteriophage Phenomena."

"The Electrical Behaviour of Bacteriophages."

Australian Journal of Experimental Biology and Medical Science, 7 (1930), 183, 199.

BURNET, F. M., and
MACNAMARA, JEAN.

"Immunological Differences between Strains of Poliomyelitis Virus."

British Journal of Experimental Pathology (1931).

(In the Press.)

BURNET, F. M.

"The Interactions of Staphylococcal Toxin, Anatoxin and Antitoxin."

Journal of Pathology and Bacteriology.

(In the Press.)

"The Flocculation Reaction with Staphylococcal Toxin."

Journal of Pathology and Bacteriology.

(In the Press.)

The Biochemical Department.

Fractionation of Snake Venoms.

Since the application of the alcohol precipitation method, applied with moderate success in the case of the venom of the death adder, proved of little value for the fractionation of other venoms, we turned our attention to filtration. Miss Freeman first experimented with pyroxylin membranes, using low pressures, and since these were unsatisfactory, Mr. Holden has carried out filtration at high pressures, using the Martin ultra-filter. In confirmation of Martin's early work, it has been shown that a moderately good separation of the thrombin and neurotoxin of black snake venom can be obtained. Preliminary observations show that the separation of the thrombin from the neurotoxin of tiger snake venom is by no means so complete.

Recent indications suggest that no filtration method is likely to give absolute separation. These studies are still in progress.

Hæmolysis by Snake Venoms.

With Dr. Kellaway and Miss Williams, Mr. Holden is investigating the protective action of sera in the hæmolysis of certain mammalian erythrocytes by snake venoms. The behaviour of lipoid-free serum proteins and of lipoids separated from the sera have been studied, and it appears that the protective effect depends upon the lipoid protein complex in the serum, and is not the sum of the actions of its constituent parts. Further work is in progress upon this problem also.

The Optical Properties of Proteins.

Mr. Holden has continued his work in collaboration with Professor Hicks, of Adelaide University, and various preparations have been made for observation of the ultra violet absorption spectra. This work has been delayed owing to the necessity for sending a portion of the apparatus to Europe for adjustment.

Mr. Holden has been engaged also in work on the quantitative separation of the globulins of ox serum and on the preparation of pseudoglobulin and serum albumin of constant optical activity.

Staphylococcus Toxin.

Some preliminary work has been carried out on the removal of impurities from crude staphylococcal toxin. The most satisfactory method for the initial stage appears to be precipitation with 70% acetone at a low temperature.

Miss Freeman is investigating the action of formaldehyde on staphylococcal toxin by estimating the rate of decrease of amino nitrogen in crude toxin, in the purer product precipitated by acetone as well as in crude nutrient fluid and nutrient fluid similarly precipitated.

The Action of Formaldehyde on Products of Peptic Hydrolysis of Proteins.

Miss Freeman is continuing her work on the action of formaldehyde on the products of enzymatic hydrolysis as revealed by their content of primary amino nitrogen.

Toxic Goitre.

Dr. Keith D. Fairley, Howard Fulford Scholar of Trinity College, is at present investigating the end results of the surgical treatment of cases of toxic goitre operated on at the Melbourne Hospital in the last nine years. The great value of the modern pre-operative treatment of iodine and the replacement of ether by nitrous oxide oxygen anæsthesia is strikingly illustrated by the lowering of the operation mortality, the halving of the period of hospitalisation, and the almost universal substitution of a one-stage subtotal thyroidectomy for the multiple stage operations formerly necessary.

Oral Sepsis.

Dr. J. M. Lewis and Dr. A. Amies are still continuing their work on pathological conditions of the alveolus and soft tissues of the mouth, and are also making a histological investigation of the teeth occurring in ovarian dermoid cysts. Owing to the scarcity of material, this last study will take some time to complete.

Work on Special Hospital Problems.

These have included studies of the sterilisation of catgut by Miss F. E. Williams, which is now in progress, and of the efficiency of antiseptics for general use, special attention being paid to the question of cost.

Mr. Holden's services have been made available for an investigation of the purity of gases used in the administration of anæsthetics.

Morbid Anatomy.

During the year 541 routine hospital autopsies were performed. Of this number, 492 were done by Dr. Wright-Smith. All autopsies were well attended by the Honorary and Resident Medical Staff, and students.

Histological examinations for the diagnosis of pathological material by Dr. C. H. Mollison and Dr. Wright-Smith numbered 763. In addition, 525 histological sections of post mortem material were made.

Demonstrations of pathological specimens were given to the Honorary Staff of the Hospital in October, 1930, and to the Post Graduate class in September, 1930.

Publications.

WRIGHT-SMITH, R. J.:

"Demonstration of Pathological Specimens."

The Melbourne Hospital Clinical Reports, November, 1930, p. 146.

"Osteogenic Sarcoma of the Tibia."

The Journal of the College of Surgeons of Australasia, March, 1931.

"Fibroma of Tendon Sheath."

The Journal of the College of Surgeons of Australasia.

(In the Press.)

Museum and Teaching.

The Museum has again been largely used by University lecturers and clinicians. Dr. C. J. O. Brown conducted pathological tutorials for the M.S. degree. Specimens have been used for demonstrations by the hospital honorary staff.

Fifty-five new specimens have been added during the past year, and many specimens remounted.

Amongst more interesting specimens were congenital malformation of the heart, cystic hygroma of neck, simple cysts of liver and kidneys, stomach in cyanide poisoning, congenital stenosis of oesophagus, novasurol colitis, glioma of corpus callosum, neurinoma of auditory nerves, sarcoma of breast, primary carcinoma of lung, endothelioma of spleen, fracture of thyroid cartilage—strangulation, cysts of semilunar cartilage, and subacute yellow atrophy of liver.

We are indebted to Dr. C. H. Mollison, Mr. B. T. Zwar, Dr. Roy Chambers and Mr. Alan Newton for the gift of rare specimens.

Numerous specimens of venomous and non-venomous Australian snakes have been added to the existing collection.

A course of lectures in Pathology was given to fourth year students, and lectures in elementary bacteriology were given to nurses by Dr. Wright-Smith.

Photography.

The macroscopic and microscopic photography was carried out by Miss Helen Wischusen.

Biochemistry, Electrocardiography and Basal Metabolism.

At the beginning of the year Miss Maudsley returned from England and took over the Basal Metabolic Rate determinations, enabling Miss Splatt to resume the whole of the biochemical work. Miss Margaret Green, a third year science student, is being trained in clinical biochemistry in the department.

The work undertaken by the Diabetic Clinic, has continued to increase this year, over five hundred blood sugar estimations having been performed on patients from the clinic as well as numerous sugar tolerance tests to establish the diagnosis of obscure cases of glycosuria.

The Renal Clinic has also provided additional material, urea concentration and blood urea tests being performed on a number of patients at regular intervals.

The past year's work clearly shows the heavy demands on the services of the biochemical department, and the pressing need of certain additions, particularly a patients' waiting room.

A detailed list of the tests performed for 1930-31 will be found in the annual report of the Melbourne Hospital.

Bacteriology and Clinical Pathology.

The increasing demands made upon the hospital accommodation for both in-patients and out-patients have again been reflected in the increase in the number of examinations made in the department during the past year; the total number was 13,378, as compared with a total of 10,890 for the previous year.

Most of the work has been of a similar nature to that performed hitherto, but in addition to the tests previously used in allergic cases, we have been trying to assess the value of the proteose described by Barber and Oriel, of Guy's Hospital, as a desensitizing agent. Miss Splatt has co-operated in the preparation of extracts of this substance.

At the suggestion of Dr. Burnet, and in conjunction with his experimental work on staphylococcal toxin, Dr. Bryce has titrated the staphylococcal antitoxin content of a number of individuals of different ages from infancy onwards. Many of these were patients at the Children's and the Queen Victoria Hospitals, and we wish to thank the medical officers of these hospitals for their helpful cooperation.

During the year we were asked to investigate, from the bacteriological aspect, an outbreak of an unusual skin condition among the infants in a charitable institution. Dr. Marion Wanliss was given facilities to carry out this work.

The testing of donors for the Red Cross Blood Transfusion Service has been continued this year, as we still need a larger number of volunteers than we have at present. This service has proved very useful to the metropolitan public hospitals, to all of which it is available. The willingness and prompt response of those donors who have been called upon have been much appreciated.

At the last annual Refresher Course arranged by the Melbourne Permanent Committee for Post Graduate Work, held in July, 1930, a demonstration of clinical pathological methods was given to members of the class by Misses Splatt and Maudsley, and Drs. Bryce and Gardner.

The hospital registrars have been given tuition in pathological methods, each one devoting part of one afternoon a week to this work.

There have been no changes in the personnel of the staff this year, but additional voluntary assistance in clerical work has been given by Miss M. Dallas Smith and Miss Leslie M. Henderson, to whom we are very grateful for their careful and accurate work.

Other routine serological work has been carried out as heretofore by Miss F. E. Williams, and has included complement fixation tests for hydatid disease, 253; Wasserman's, 398; bilharzia, 9; and for gonococcal disease, 40.

The Casoni test has been extensively used. Dr. Wright-Smith has carried out 224 tests, with 34 positive and 190 negative results.

The Library.

Our thanks for the gift of journals and books are due to the following:—

Dr. S. O. Cowen, Miss Danks, Dr. K. D. Fairley, Dr. C. H. Kellaway, L.Académie Royale de Médicine de Belgique, the Commonwealth Department of Health, the Council of Scientific and Industrial Research, the Government Institute for Infectious Diseases of the Tokyo Imperial University, the National Institute of Medical Research (London), the Rockefeller Institute (New York), la Société royale des Sciences Médicales, Cancer Research Committee (Sydney), Universitets Biblioteket (Lund, Sweden).

Walter and Eliza Hall Institute of Research in Pathology and Medicine.

FINANCIAL STATEMENT FOR THE YEAR ENDED 30th JUNE, 1931.

Receipts.

To Balance brought forward from 30th June, 1930				£4,675	0 10
Trustees of Walter & Eliza Hall Trust	£3,200	0	0		
University of Melbourne	1,500	0	0		
Commonwealth Grant for Research Work	700	0	0		
Felton Bequest Committee	270	0	0		
Trustees of Estate Late Henry Berry	25	0	0		
White Hand Social Party	1	1	0		
Fees Received for Tests, etc.	363	8	3		
Interest on Investments	200	18	9		
				6,260	8 0
				£10,935	8 10

Expenditure.

By Apparatus	£115	8	9		
Fittings and Equipment	10	10	6		
Repairs to Apparatus	8	6	3		
Repairs to Buildings	13	11	9		
Materials	775	0	2		
Publications	72	17	5		
Salaries and Wages	4,930	14	6		
Sundries	157	16	10		
				£6,084	6 2
Advance to Bio-chemical A/c.				305	8 7
Balance—					
Fixed Deposits, 5¼%	£2,000	0	0		
Credit Foncier Debenture Stock, 5¼%	875	0	0		
Cash with Agent-General, London	50	8	4		
Secretary's Advance A/c., E., S. & A. Bank Ltd.	100	0	0		
Bank N.S.W. Current A/c.	1,520	5	9		
				4,545	14 1
				£10,935	8 10

BIO-CHEMICAL DEPARTMENT—ENDOWMENT ACCOUNT.

To Balance brought forward from 30th June, 1930			£8,591	10	3	By Bank Charges for Collecting Deb. Int.		£1 12 6
Interest on Investments			505	0	6	Transfer to Bio-chemical Department		503 8 0
						Balance—		
						C'wealth Ins. Stock, 5¼%	£500 0 0	
						C'wealth Ins. Stock, 6%	140 0 0	
						City of Melb. Debs., 5½%	807 12 2	
						M. & M. Board Works Ins. Stock, 4%	1,218 4 3	
						M. & M. Board Works Ins. Stock, 6%	1,952 11 1	
						Melb. Harbour Trust Debs., 5⅝%	500 0 0	
						Credit Foncier Deb. Stock	621 5 0	
						Mortgage—Anthony, 6%	2,550 0 0	
						Mortgage—Montgomery 7%	300 0 0	
						Bank N.S.W.	1 17 9	
								8,591 10 3
			£9,096	10	9			£9,096 10 9

LIBRARY ACCOUNT.

To Balance brought forward from 30th June, 1930			£2,000	0	0	By Books, Journals and Bookbinding		£108 2 2
Interest on Investments			128	15	0	Balance—		
						C'wealth Treasury Bond, 5¾%, 1940	£1,000 0 0	
						Mortgage—Montgomery	1,000 0 0	
								2,000 0 0
						Bank N.S.W.		20 12 10
			£2,128	15	0			£2,128 15 0

BIO-CHEMICAL DEPARTMENT.

To Balance brought forward from 30th June, 1930	£220	9	2	By Salaries and Wages	£1,061	5	3
Donations—				Materials and Apparatus	10	15	7
Mrs. Alcock	20	0	0	Sundries	2	9	11
Mr. J. A. Levey	2	2	0				
Mr. Tweddell	3	3	0				
Estate T. J. Sumner, decd.	20	0	0				
Int. Trans. from Endowment A/c.	503	8	0				
	£769	2	2				
Advance from Working A/c.	305	8	7				
	£1,074	10	9		£1,074	10	9

Auditor's Certificate.

I have to report that I have completed the Audit of the Books and Accounts of the Institute for the period ended 30th June, 1931. I have verified all receipts and have had vouchers produced for all disbursements. All information and explanations required have been given. The Statement is a correct statement of Receipts and Expenditure as revealed by the books of the Institute.

Dated 8th July, 1931.

W. M. JARVIE, F.C.A. (Aust.), Auditor.